

## Relation between the increase of circulating CD3<sup>+</sup>CD57<sup>+</sup> lymphocytes and T cell dysfunction in recipients of bone marrow transplantation

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(Accepted for publication 16 May 1990)

### SUMMARY

Some patients undergoing bone marrow transplantation (BMT) persistently present increased proportions of circulating CD57<sup>+</sup> T cells. We analysed the cell surface phenotype in peripheral blood mononuclear cells (PBMC) from 69 allogeneic and 11 autologous BMT recipients. In parallel samples from 49 patients, the proliferative response to T cell mitogens was assessed, either in the presence or absence of exogenous interleukin-2 (IL-2). PBMC samples from long-term allogeneic BMT patients with increased proportions of CD57<sup>+</sup> cells displayed significantly ( $P < 0.001$ ) lower proliferative responses, compared with samples from patients with normal proportions of CD57<sup>+</sup> cells and from healthy subjects. Elimination of the CD57<sup>+</sup> population by C'-dependent lysis did not normalize the proliferative response. After positive selection by cell sorting, CD57<sup>+</sup> cells responded poorly, but in the presence of IL-2 the proliferation appeared to be similar to that displayed by the CD57<sup>-</sup> subset and still suboptimal compared with normal controls. These data suggest that the hyporesponsiveness to mitogenic stimuli in the presence of exogenous IL-2 of PBMC from allogeneic BMT recipients cannot be simply attributed either to the putative suppressor activity of CD57<sup>+</sup> cells, or to a poor proliferative capacity of this subset. Supporting this notion we report that PBMC from long-term autologous BMT recipients containing high proportions of CD57<sup>+</sup> T cells respond normally to T cell mitogens.

**Keywords** bone marrow transplantation CD57<sup>+</sup> cells T cell dysfunction

### INTRODUCTION

Different alterations related to the phenotype and function of circulating T lymphocytes have been described during the immune reconstitution process following bone marrow transplantation (BMT). Some of the remarkable phenotypic features reported are a reversed CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio (reviewed by Witherspoon, Lum & Storb, 1984; Lum, 1987) and increased proportions of HLA-DR<sup>+</sup> and CD8<sup>+</sup>/CD57<sup>+</sup> circulating T cells (Leroy *et al.*, 1986; Forman *et al.*, 1986). With regard to T cell function, low proliferative responses to mitogens, allogeneic cells and antigens (Lum, 1987), as well as a reduction in the frequency of T cells with clonogenic potential (Rozans *et al.*, 1986; Viale *et al.*, 1989) have been reported. In these observations the incidence of graft-versus-host disease (GVHD) delayed re-acquisition of normal T cell function.

Several alterations have been observed to underlie the T cell dysfunction, such as a depressed interleukin-2 (IL-2) synthesis

(Azogui, Gluckmann & Fradelizi, 1983) and defective IL-2 receptor expression capacities (López-Botet *et al.*, 1987). Moreover, we have recently shown a deficient protein kinase C-dependent Na<sup>+</sup>/H<sup>+</sup> exchanger activity in phenotypically mature circulating T lymphocytes from BMT recipients which displayed low proliferative responses to T cell mitogens (Izquierdo *et al.*, 1989), thus suggesting that these cell populations might present intrinsic metabolic defects. However, increased non-specific suppressor activity exerted by CD8<sup>+</sup> cells has been proposed to interfere with the *in vitro* mitogenic T cell response (Harada *et al.*, 1986). In the same line, it has been reported that high levels of CD3<sup>+</sup>CD8<sup>+</sup>CD57<sup>+</sup> cells, a minor lymphocyte subset in healthy individuals (Abo, Cooper & Balch, 1982), suppress the T cell-dependent immunoglobulin synthesis in B lymphocytes after marrow grafting (Leroy *et al.*, 1986). Regarding the T cell dysfunction associated to BMT, the role exerted by that particular cell subset remains uncertain.

Here we report an apparent correlation between the depressed T cell proliferative response and the increased proportions of CD57<sup>+</sup> T cells in allogeneic BMT recipients. On the basis of negative and positive selection experiments per-

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formed with this subpopulation, we conclude that the elevated percentages of CD57<sup>+</sup> cells do not account for the T cell defective proliferation. Supporting this view we have observed that, in contrast to allogeneic BMT, samples obtained from long-term autologous BMT recipients with high proportions of CD8<sup>+</sup>CD57<sup>+</sup> T cells exhibit a normal response to T cell mitogens.

## SUBJECTS AND METHODS

### Patient population

Sixty-nine patients who had undergone therapeutic allogeneic BMT were studied. Fifty-nine were transplanted for haematologic malignancies and 10 for aplastic anaemia. Pretransplant conditioning and prophylaxis of GVHD was carried out as described (Feig *et al.*, 1983; Storb *et al.*, 1986). In seven cases, T cell depletion with the monoclonal antibody Campath-1 (Waldman *et al.*, 1984) for prophylaxis of GVHD was performed. Additionally, 11 autologous BMT recipients were included in our studies. Autologous BMT leukaemia patients were treated with a combination of high-dose cyclophosphamide and fractionated total body irradiation as described (Appelbaum & Buckner, 1986).

### Cells

Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood by centrifugation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden). In the negative selection experiments, PBMC were incubated at 10<sup>7</sup> cells/ml with HNK-1 hybridoma culture supernatant (100 µl for 10<sup>6</sup> cells) for 1 h at 4°C. After washing, cells were resuspended in diluted (1/2) rabbit complement (Boehringer, Mannheim, FRG) and incubated for 60 min at 37°C. Control (untreated) cells were incubated in parallel with an irrelevant antibody (X63) before the addition of complement. The percentages of residual CD57<sup>+</sup> cells after treatment were estimated by flow cytometry and were < 5% in every case.

### Cell sorting

For sterile immunofluorescence sorting of CD57<sup>+</sup> and CD57<sup>-</sup> subpopulations, cells were pre-incubated with HNK-1 monoclonal antibody as described above, then with the FITC-labelled goat anti-mouse immunoglobulin (Dako, High Wycombe, UK). Cell sorting was performed in an EPICS-C (Coulter, Hialeah, FL). Sorted cells were collected in fetal calf serum (FCS) and washed before proliferative assays. Total population was manipulated under identical conditions.

### Flow cytometry analysis

For phenotypic evolution analyses, patients were grouped according to the time after BMT. It is noteworthy that several cases were included in different groups according to the time elapsed when the different tests were performed. Data corresponding to 36 patients (eight, 13 and 15 patients from groups C, D and E, respectively) were obtained from frozen PBMC. As previously described (López-Botet *et al.*, 1987), cells were labelled by an indirect immunofluorescence technique using as second reagent FITC-labelled rabbit anti-mouse immunoglobulin F(ab')<sub>2</sub> and analysed by flow cytometry. Monoclonal antibodies employed included SPV-T3b anti-CD3 (Spits *et al.*, 1985), B.9.4.2. anti-CD8 (Malissen *et al.*, 1982), Hp2.6 anti-

CD4 (Carrera *et al.*, 1986), B73.1 anti-CD16 (Perussia *et al.*, 1983), HNK-1 anti-CD57 (Abo & Balch, 1981). Cells displaying fluorescence intensity above the negative control were considered positive. For two-colour immunofluorescence analysis, mononuclear cells were previously labelled with HNK-1 and second FITC reagent, then incubated additionally with either Leu 2a (anti-CD8), Leu 3a (anti-CD4) or Leu 4 (anti-CD3) monoclonal antibodies conjugated with phycoerythrin (Becton Dickinson, Mountain View, CA).

### Proliferation assays

Cells were cultured in RPMI 1640 (Flow Laboratories) supplemented with 1% antibiotics (Flow), 1% L-glutamine (Flow) and 10% FCS (Gibco, Grand Island, NY). Triplicate samples were seeded (1 × 10<sup>5</sup> cells/well) in 96-well, U-bottomed microtitre plates (Costar, Cambridge, MA), either in medium alone, or in the presence of a suitable concentration of either 1% PHA-M (Difco); or anti-CD3 coupled to CNBr-Sephrose beads (CD3-Seph) according to the manufacturer's instructions (Pharmacia) as described (López-Botet *et al.*, 1987). Parallel cultures were supplemented with 10 U/ml of recombinant IL-2 (Biogen, Geneva, Switzerland). All cultures were set up in triplicate and maintained for 96 h at 37°C in a CO<sub>2</sub> incubator. During the last 18 h <sup>3</sup>H-Thymidine (Amersham) incorporation was determined. No proliferative assays were performed with frozen PBMC samples from BMT recipients.

## RESULTS

### Phenotypic analysis after allogeneic BMT

Tests were performed in PBMC from allogeneic BMT recipients that were divided in five groups (A–E) according to the time elapsed after BMT. PBMC were analysed by indirect immunofluorescence and flow cytometry for the expression of different cell membrane markers. In agreement with previous studies, we observed significantly reduced proportions of CD4<sup>+</sup> and increased percentages of CD8<sup>+</sup> cells as compared with control PBMC samples (Table 1). Moreover, in contrast with the normal proportions of CD16<sup>+</sup> cells, high levels of CD57<sup>+</sup> lymphocytes were often detected, and this alteration appeared to persist in groups from long-term BMT recipients.

For the additional characterization of CD57<sup>+</sup> cells, two-colour immunofluorescence analyses were performed in samples containing high percentages of CD57<sup>+</sup> cells (> 30%), and the proportions of lymphocytes coexpressing CD57 with CD3, CD4, and CD8 antigens were evaluated. As shown in Table 2, the CD57 antigen appeared mainly distributed in CD3<sup>+</sup>CD8<sup>+</sup> cells.

### Relation between the proportions of CD57<sup>+</sup> cells and T cell dysfunction

Since normal proportions of T cells with a mature phenotype (CD3<sup>+</sup>) were detected in all cases of long-term BMT recipients (Table 1), we directly compared the proliferative responses to anti-CD3-Sephrose in the presence or absence of exogenous IL-2, of PBMC isolated from 38 BMT recipients from groups C, D and E (see Table 1) and from healthy control subjects (*n* = 45). Samples were classified according to the percentages of CD57<sup>+</sup> cells (group I, CD57<sup>+</sup> cells ≤ 30%; group II, CD57<sup>+</sup> cells > 30%). The cut-off, 30%, was arbitrarily established as the mean ± (2 × s.d.) of the percentage of CD57<sup>+</sup> cells detected in

**Table 1.** Flow cytometry analysis of peripheral blood mononuclear cells\* from allogeneic bone marrow transplantation (BMT) recipients

Cell membrane markers	Group A (n = 18)	Group B (n = 21)	Group C (n = 27)	Group D (n = 25)	Group E (n = 17)	Control (n = 11)
CD3	58 ± 15†	64 ± 13 (NS)	69 ± 11 (NS)	65 ± 11 (NS)	64 ± 10 (NS)	68 ± 9
CD4	17 ± 6‡	21 ± 8‡	22 ± 9‡	28 ± 9‡	39 ± 11 (NS)	43 ± 8
CD8	46 ± 18‡	40 ± 15‡	49 ± 15‡	41 ± 10‡	34 ± 9†	25 ± 6
CD57	30 ± 14‡	34 ± 14‡	42 ± 16‡	37 ± 13‡	27 ± 15†	14 ± 7
CD16	16 ± 12 (NS)	10 ± 10 (NS)	8 ± 6 (NS)	6 ± 5†	10 ± 8 (NS)	10 ± 6

Patients were grouped according to post-transplant period as follows: group A, 2–3 months; group B, 4–6 months; group C, 9–12 months; group D, 18–36 months; and group E, > 36 months after transplantation at the time of testing.

\* Cells from allogeneic BMT patients or healthy subjects (control) were labelled with different monoclonal antibodies and analysed by flow cytometry. Results are expressed as mean percentage ± s.d. of positively stained cells.

†  $P < 0.05$ ; ‡  $P < 0.001$ ; NS, not significant (Mann–Whitney *U*-test).

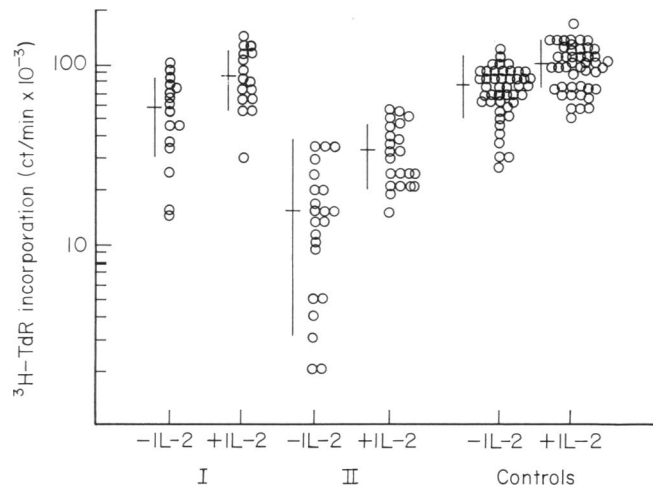
**Table 2.** Co-expression of CD57 antigen with CD3, CD4 and CD8

Cell membrane markers	Controls (n = 5)	Allogeneic BMT recipients (n = 12)
CD3	69 ± 3	73 ± 11
CD4	40 ± 12	22 ± 8
CD8	24 ± 3	57 ± 11
CD57	10 ± 4	51 ± 11
CD57CD3	6 ± 3	42 ± 9 (82)*
CD57CD4	1 ± 1	9 ± 5 (17)
CD57CD8	4 ± 2	38 ± 9 (75)

Peripheral blood mononuclear cells from allogeneic bone marrow transplantation (BMT) recipients containing > 30% of CD57<sup>+</sup> cells and healthy controls were labelled with different monoclonal antibodies and analysed by flow cytometry. Two-colour immunofluorescence analysis was performed as indicated in Subjects and Methods. Results are expressed as percentages of positively stained cells (mean ± s.d.).

\* Percentages of double-stained cells referred to the CD57<sup>+</sup> cell population.

the control group (see Table 1). As shown in Fig. 1, the proliferative response to anti-CD3–Sepharose of PBMC samples from BMT recipients displaying high proportions of CD57<sup>+</sup> cells (group II) was significantly decreased ( $P < 0.001$ ) compared with the responses of both the control group and the group of BMT recipients with 'normal' proportions of CD57<sup>+</sup> cells (group I). It is noteworthy that the addition of exogenous IL-2 only partially restored the mitogenic response of T cells ( $P < 0.001$ ). Similar results were obtained using phytohemagglutinin (PHA) as a mitogenic stimulus (not shown). These results suggested a possible relation between the high percentages of CD57<sup>+</sup> cells and the low proliferative responses to mitogenic stimuli. In fact, when data from BMT recipients were pooled and lineal regression analysis was performed, relating proliferation to anti-CD3–Sepharose in the presence or absence of IL-2



**Fig. 1.** Relation between the proliferative response to anti-CD3–Sepharose of peripheral blood mononuclear cells (PBMC) from allogeneic long-term bone marrow transplantation (BMT) recipients and the percentage of CD57<sup>+</sup> cells. PBMC from long-term allogeneic BMT recipients were cultured ( $10^5$  cells/well) in the presence of anti-CD3–Sepharose and  $^3\text{H}$ -TdR incorporation was evaluated after 3 days of culture as indicated in Subjects and Methods. Replicate cultures were costimulated with IL-2 (10 U/ml). Patients were grouped according to the percentages of CD57<sup>+</sup> cells (group I,  $n = 16$ , CD57<sup>+</sup> cells  $\leq 30\%$ ; group II,  $n = 22$ , CD57<sup>+</sup> cells  $> 30\%$ ). Statistically significant differences ( $P < 0.001$ ) were only obtained when comparing the proliferation corresponding to mitogen-stimulated cultures from group II with those of group I and the controls ( $n = 45$ ). For each group, the mean ± s.d. of  $^3\text{H}$ -TdR incorporation is represented.  $^3\text{H}$ -TdR uptake (mean ± s.d. ct/min  $\times 10^{-3}$ ) corresponding to unstimulated cultures was  $1.6 \pm 1.1$  without IL-2 and  $7.8 \pm 5.9$  with IL-2 for patients ( $n = 38$ ); and  $2.5 \pm 1.4$  without IL-2 and  $11.2 \pm 7.8$  with IL-2 for healthy controls ( $n = 45$ ).

versus CD57 proportions, a significant correlation was revealed ( $r = -0.771$ ;  $P < 0.01$  without IL-2;  $r = -0.779$ ,  $P < 0.01$  with IL-2). Additionally, we performed a non-parametric test of independence and both variables appeared related ( $P < 0.001$ ) ( $r_s = -0.793$  without IL-2;  $r_s = -0.800$  with IL-2).

**Table 3.** Proliferative response of CD57<sup>+</sup> cells in peripheral blood mononuclear cells (PBMC) from bone marrow transplantation (BMT) recipients

BMT patient*	Proliferative response (ct/min × 10 <sup>-3</sup> )†			
	With IL-2		Without IL-2	
	Total	CD57 <sup>+</sup>	Total	CD57 <sup>+</sup>
1	5.8	10.4	23.2	36.4
2	9.1	13.9	23.3	40.5
3	9.2	19.2	30.4	48.3
4	13.1	20.5	48.1	56.2
5	22.0	32.9	41.2	53.9
Controls (n = 3)	68.5 ± 10.4	73.1 ± 13.9	79.5 ± 28.1	104.1 ± 37.2

\* PBMC from five allogeneic long-term BMT recipients with percentages of CD57<sup>+</sup> cells > 30%.

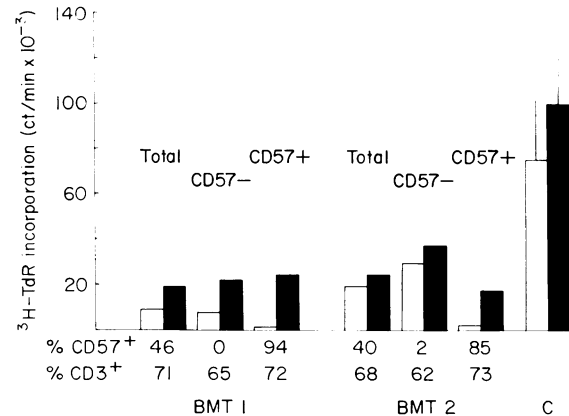
† Total PBMC and PBMC depleted of CD57<sup>+</sup> cells (CD57<sup>-</sup>) by means of treatment with HNK-1 monoclonal antibody plus C' were stimulated with anti-CD3-Seph in the presence or absence of IL-2 as indicated in Subjects and Methods. In all cases the percentages of CD3<sup>+</sup> cells were comparable. The proportions of residual CD57<sup>+</sup> cells after the incubation with complement were < 5% in every experiment. Samples from healthy subjects (controls) were tested in parallel.

#### Role of CD8<sup>+</sup>CD57<sup>+</sup> cells in the regulation of the T cell proliferative response

To evaluate the possible regulatory role exerted by the CD8<sup>+</sup>CD57<sup>+</sup> subpopulation on T cell function we initially conducted negative selection experiments by using the anti-CD57 monoclonal antibody (HNK-1) and C', assessing the proliferative response of PBMC samples from several allogeneic BMT recipients depleted of CD57<sup>+</sup> cells. Table 3 illustrates the results of tests performed in five cases from group II (CD57<sup>+</sup> cells > 30%). Although the CD57<sup>-</sup> subset (< 5% of CD57<sup>+</sup> cells) did proliferate better than the total population, elimination of CD57<sup>+</sup> cells did not restore the proliferative response to anti-CD3 up to the levels obtained in the control group, even in the presence of exogenous IL-2. Similar results were obtained after PHA stimulation (not shown). These data indicate that the hyporesponsiveness to mitogenic stimuli of PBMC from long-term BMT cannot be simply attributed to an active *in vitro* suppressor activity exerted by CD57<sup>+</sup> cells.

To determine whether the low proliferative response could be due to an inability of the CD57<sup>+</sup> population itself to respond to mitogenic stimuli, we performed positive selection experiments. The response induced by anti-CD3-Seph in immunofluorescence-sorted CD57<sup>+</sup> cells was compared with that of the total population and CD57<sup>-</sup> cells. The results of two representative experiments performed in allogeneic BMT recipients are shown in Fig. 2. CD57<sup>+</sup> T cells proliferated poorly in the absence of exogenous IL-2 compared with the whole population and the CD57<sup>-</sup> subset. However, in the presence of IL-2 the proliferative response of both the CD57<sup>+</sup> and CD57<sup>-</sup> cells appeared to be similar and still clearly diminished compared with that of controls.

Altogether, these data suggested that the hyporesponsiveness to mitogenic stimuli of PBMC from allogeneic BMT



**Fig. 2.** Proliferative capacity of CD57<sup>+</sup> cells from allogeneic bone marrow transplantation (BMT) recipients. CD57<sup>+</sup> and CD57<sup>-</sup> cells from two allogeneic BMT recipients (BMT 1 and BMT 2) were selected by cell sorting after staining with the HNK-1 mAb. The proliferative response to anti-CD3-Seph with (■) or without IL-2 (□) of sorted cells was compared with that of unfractionated (total) peripheral blood mononuclear cell (PBMC) samples. Final percentages of CD57<sup>+</sup> and CD3<sup>+</sup> cells are indicated. As reference, the proliferation (mean ± s.d.) corresponding to PBMC samples from a group of 45 healthy individuals is shown (C).

**Table 4.** Expression of CD57 antigen and proliferative response of peripheral blood mononuclear cells (PBMC) from long-term autologous bone marrow transplantation (BMT) recipients

BMT patient*	CD57 <sup>+</sup> cells (%)†	† Proliferative response (ct/min × 10 <sup>-3</sup> )‡	
		With IL-2	Without IL-2
1	14	92.8	113.5
2	19	51.3	70.8
3	11	107.2	116.7
4	27	72.4	89.1
5	10	44.3	60.7
6	13	72.5	93.4
7	41	79.8	83.4
8	42	87.0	96.2
9	32	56.4	77.0
10	59	64.3	66.5
11	35	108.6	107.1
Controls (n = 45)	14 ± 7	75.2 ± 25.8	98.5 ± 27.5

\* Long-term autologous BMT recipients.

† By flow cytometry.

‡ PBMC were stimulated with anti-CD3-Seph as indicated in Subjects and Methods in the presence or absence of exogenous IL-2.

recipients cannot be attributed merely to the putative suppressor activity of CD57<sup>+</sup> subset or to a poor proliferative capacity of these cells.

*Expression of CD57 and T cell proliferative response in autologous BMT recipients*

Phenotypic and functional studies were performed in PBMC from 11 long-term autologous BMT recipients. Persistent increased proportions of CD57<sup>+</sup> T cells were observed in several cases, similar to the data obtained in allogeneic BMT recipients. As shown in Table 4, samples with increased proportions of CD57<sup>+</sup> cells displayed a normal proliferative response compared with the control group. These results support that an expanded CD8<sup>+</sup>CD57<sup>+</sup> subset is not necessarily associated to a depressed mitogenic response of PBMC from BMT recipients.

## DISCUSSION

CD57 is a non-lineage-restricted surface antigen whose biological function remains unknown. A minor subset of normal T lymphocytes coexpresses CD57 with either CD4 or CD8 differentiation markers (Abo *et al.*, 1982). Whether the CD3<sup>+</sup>CD57<sup>+</sup> subset presents specific functional properties different from those displayed by CD57<sup>-</sup> T cells is uncertain. CD57<sup>+</sup> T cells can be activated to express cytotoxic effector function (Divine *et al.*, 1988). Several *in vitro* studies have ascribed to the CD57<sup>+</sup>CD8<sup>+</sup> T subset a suppressor regulatory role. CD57<sup>+</sup> T cells from normal subjects were shown to inhibit the *in vitro* immunoglobulin synthesis upon polyclonal stimulation of B cells (Tilden, Abo & Balch, 1983) and similar data were obtained when CD57<sup>+</sup> T cells were derived from allogeneic BMT recipients (Leroy *et al.*, 1986). More recently, an inhibition of *in vitro* human haemopoietic cell growth mediated by CD57<sup>+</sup> T cells from BMT recipients has been reported (Vinci *et al.*, 1988). Finally, Tilden *et al.* (1983) provided data indicating that the CD57<sup>+</sup> T cells may partially inhibit the T cell proliferative response in mixed lymphocyte culture. Since some long-term BMT recipients display high proportions of that particular subset, we have investigated the possible relation between this feature and the persistence of the T cell dysfunction associated to BMT. Here we observed an apparent correlation between defective T cell proliferative response to mitogenic stimuli and increased proportions of CD8<sup>+</sup>CD57<sup>+</sup> T cells in PBMC isolated from long-term allogeneic BMT recipients. To clarify the role exerted by CD57<sup>+</sup> cells on the T cell dysfunction we conducted in several cases negative and positive selection experiments analysing the response of CD57<sup>+</sup> and CD57<sup>-</sup> cells. Our results indicate that the hyporesponsiveness to mitogenic stimuli of PBMC isolated from long-term BMT recipients could not be attributed to a direct 'suppressor' effect mediated by the CD8<sup>+</sup>CD57<sup>+</sup> subset, since its elimination by C' dependent lysis or cell sorting partially increased but did not restore to normal levels the response of the CD57<sup>-</sup> population. However, we observed that CD57<sup>+</sup> cells from BMT recipients proliferated in the presence of exogenous IL-2 comparably to the CD57<sup>-</sup> subset, thus indicating that the defective proliferation of the total population could not be ascribed to an inability of the CD57<sup>+</sup> subset to respond to mitogenic stimuli, provided exogenous IL-2 was supplied. Altogether, our results indicate that the T cell dysfunction does not appear to be exclusively related to the increased proportion of CD57<sup>+</sup> cells, and that both phenomena are simply concomitant in some long-term allogeneic BMT recipients. Supporting this notion we report that in several autologous BMT recipients high levels of

CD8<sup>+</sup>CD57<sup>+</sup> cells did co-exist with a normal proliferative response to mitogenic stimuli.

Several studies have shown that increased proportions of circulating CD57<sup>+</sup> T cells correlate with the incidence of cytomegalovirus (CMV) infections both in normal subjects and in BMT recipients (Forman *et al.*, 1986; Gratama *et al.*, 1988). On the other hand, CMV infections have been proposed to favour the development of GVHD (de Gast *et al.*, 1987). In our experience, a persistent T cell dysfunction after allogeneic BMT is mainly associated with the incidence of either acute or chronic GVHD, as previously reported by others (reviewed by Lum, 1987). Altered T cell function can also be observed in some long-term allogeneic recipients without overt chronic GVHD who do not receive immunosuppressive therapy. Nevertheless, the possibility that administration of cyclosporine A (CyA) during the first 6 months for GVHD prevention may interfere with T cell ontogeny and delay immune reconstitution cannot be entirely excluded. A possible explanation for the apparent relation between low proliferative response to mitogens and increased proportions of CD57<sup>+</sup> cells observed in our study is the higher frequency of CMV infections in the group of patients undergoing GVHD which, in addition, have developed a T cell immunodeficiency. Nevertheless, it is noteworthy that in agreement with others (Leroy *et al.*, 1986) we have not found a correlation between the levels of CD57<sup>+</sup> cells and the incidence of GVHD, and our data in autologous BMT recipients support that both phenomena are not necessarily related. To understand the pathogenetic mechanisms leading to the often dramatic and persistent expansion of the CD57<sup>+</sup> subset in BMT recipients, further studies are required. No data are so far available regarding the antigen specificities of CD57<sup>+</sup> T cells, and thus analysis of the pattern of T cell receptor gene rearrangements in clones derived from that population could provide information on its possible representation of an oligoclonal expansion, reflecting a specific response to CMV infection.

## ACKNOWLEDGMENTS

This work was supported by grants from Insalud (FISS 88/1737). We thank Mr D. Hernández and Mr M. Vitón for technical assistance.

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